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Cell Behavior and Signal Molecule Involvement in a Case Study of Malignant Histiocytosis: A Negative Model of Morphine as an Immunoregulator

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In a patient diagnosed with histiocytic medullary reticulosis (HM), we examined immunocytes for their responsiveness towards known signaling molecules. Both the granulocytes and monocytes were found to exhibit a high level of spontaneous activation (96% compared to normal cells 7%; $P < 0.001$). These cells could not be downregulated when exposed to morphine. Following patient treatment with doxorubicin and cyclophosphamide, the immunocytes still exhibited a high spontaneous activation. They responded to morphine exposure in vitro with a cell rounding and becoming immobile for only 20 min whereas normal cells would remain round and immobile for up to 1–2 h. An examination of the plasma from the HM patient revealed that monocyte colony stimulating factor (MCSF) levels were elevated (6.4 and 5.78 compared to a control range of 1–1.75 ng/ml). In the HM patient, the immunocytes did not express the opiate selective and opioid peptide insensitive receptor, μ3, supporting the lack of opiate action. Given this finding, we incubated normal monocytes with MCSF and found that it significantly reduced the μ3 Bmax. Given the role of intracellular calcium in the activation process of immunocytes, we examined the action of various calcium channel blockers for their ability to inhibit the activated HM monocytes. The agents (nimodipine, cardiazem, and verapamil; 10^{-9} M) were able to inhibit the HM-associated chemokinesis. Taken together, the data indicate that in the HM patient the immunocytes appear to be overactivated because stimulatory molecules are high and have the ability to downregulate the normal ''braking'' process. Additionally, the data indicate that MCSF deregulation may be involved as an initiating factor for this disorder. Am. J. Hematol. 56:197–205, 1997. © 1997 Wiley-Liss, Inc.

Key words: medullary reticulosis; morphine; granulocytes; monocytes; mu3

INTRODUCTION

The term ''histiocytic medullary reticulosis'' (HM) was coined by Bodley and Smith in 1939 [1]. In their description of these patients, a rapid fatal course characterized by fever, lymphadenopathy, hepatosplenomegally, anemia, and leukopenia was noted, yet it was not clear that the cause was a neoplastic one. The authors made a point of avoiding the term malignant for a condition described as ''proliferation of erythrophagocytic histiocytes and their precursors.'' Rappaport in 1966 introduced the term malignant histiocytosis in an attempt to further define this rare rapidly fatal disease [2]. The debate about whether this disease is malignant from the onset or represents a malignant degeneration or a clinically fulminant but not neoplastic process of an initially

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benign condition is, even today, not resolved. Neither is it entirely clear how to categorize this disease despite considerable progress in the classification of other hemopoietic proliferative disorders [3].

In regard to HM, from a histological perspective, hematophagocytosis can be carried out not only by macrophages or tissue histiocytes, but also by hematopoietic and non-hematopoietic malignant tumor cells [4]. It is well established that malignant lymphomas can recruit reactive cells [4]. In Hodgkin's disease, for instance, reactive lymphocytes, macrophages, and leukocytes can be the predominant cells [5]. This is far less typical for malignant histiocytosis. One possible explanation for this recruitment is the secretion of cytokines into the microenvironment by the malignant cells. In large cell lymphoma, this may occur due to modifications of c-fms, colony stimulating factor (CSF-1), and/or monocyte colony stimulating factor (MCSF) and tumor necrosis factor-alpha (TNA-α) with modulation of the chromosomal 5q35 breakpoint region genes [6]. TNF levels have been found useful in predicting the severity and prognosis of both malignant histiocytosis and virus-associated hemophagocytic syndrome (VAHS). This information can be used successfully to monitor the progression of the disease and therapy [7].

Given this background, and based on the recent work regarding the role of neuropeptides, cytokines, and opiate alkaloids in immunoregulation and autoimmunoregulation [8–10], we examined immunocytes and serum obtained from a patient diagnosed with autopsy confirmed malignant histiocytosis, to determine if deficits existed in their immunocyte responsiveness to naturally occurring immunocyte-suppressing substances [8–13]. We report, for the first time, that both granulocytes and monocytes obtained from this patient are dysfunctional, based on their level of spontaneous activation and lack of appropriate responses to known immunosuppressory agents. Further, in vitro analysis of normal immunocytes exposed to MSCF mimics, in part, the cell behavior and biochemical changes found in cells obtained from this patient.

CASE REPORT

A sixty-five-year-old male was admitted with abdominal pain of 5 days duration, and fever, and was found to have a platelet count of 17,000 per microliter. He had previously been in good health, except for a past history of osteoarthritis treated with naproxen, and a history of Guillain-Barre syndrome 12 years prior to admission.

Initial physical examination was significant for hepatosplenomegaly. The white blood cell count was 13.2×10^3 per microliter, with 50% segmented neutrophils, 14% lymphocytes, and 16% monocytes. Hemoglobin was 16.1 g per deciliter. The blood smear showed atypical monocytes, large platelets, and schistocytes. Creatinine was 2.2 mg per deciliter, BUN was 141 mg per deciliter, total bilirubin was 4.4 mg per deciliter with 1.6 mg per deciliter direct. AST was 28 IU per liter, ALT was 30 IU per liter, and LDH was 521 IU per liter. PT was 13.8 sec and PTT was 33.1 sec.

Bone marrow was aspirated with difficulty; aspiration and biopsy revealed extensive replacement of the normal hematopoietic elements by pleiomorphic, immature-appearing monocytoid cells, and numerous instances of hemophagocytosis. The infiltrating population exhibited nonspecific esterase staining, with partial fluoride inhibition. Cytogenetics were normal, demonstrating a karyotype of 46, XY. Flow cytometric immunophenotyping of the patient's blood identified a population of large, mononuclear cells comprising 56% of circulating leukocytes and co-expressing CD45, CD14, CD11b, CD11c, CD15, CD34, and CD33. A blood specimen sent to a research laboratory showed markedly elevated levels of MCSF (patient 6.4 ng per ml, control 1.0 ng per ml), and G-CSF (patient 350.3 pg per ml, control 53.5 pg per ml). Other measured cytokine levels included TNF (patient 30.3 pg per ml, control <16 pg per ml), SCF (patient 1.4 ng per ml, control 1.1 pg per ml), GMCSF (patient and both <8 pg/ml), and II-1beta (patient and control both <8 pg per ml). Southern hybridization analysis of peripheral blood mononuclear cells showed no rearrangement of immunoglobulin or T-cell receptor genes. The diagnosis of histiocytic medullary reticulosis was made.

Initial attempts to ameliorate severe and transfusion-refractory thrombocytopenia with corticosteroids and intravenous gammaglobulin were unsuccessful. Combination chemotherapy with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) was begun. Concomitantly, the patient received 20 cGy splenic radiotherapy in eight fraction, with an approximately 50% reduction in spleen size; this was associated with a rise in platelet count, and improvement in response to platelet transfusion. The first three cycles of chemotherapy were administered with 50–80% of standard doses of doxorubicin and cyclophosphamide, followed by full doses of the same regimen administered for a total of seven cycles.

Cytogenetics performed on a bone marrow aspirate obtained after cycle 1 indicated 46, XY, del(13), (q12q14). Flow cytometric immunophenotyping of marrow cells at that time confirmed the phenotype originally obtained on peripheral blood. Repeat cytogenetic studies on bone marrow after cycle 3 were normal, and Southern hybridization analysis of marrow mononuclear cells again showed no immunoglobulin or T-cell receptor gene rearrangements.

The patient experienced a progressive response, achieving normal white blood cell and platelet counts,

and a rising hemoglobulin, at the time therapy was stopped 6 months after presentation. At this time, marrow examination showed hypercellularity, with scattered atypical monocytoid cells, normal cytogenetics, and germ line immunoglobulin and T-cell receptor genes by Southern hybridization. A specimen of blood was stored at this time in the original research laboratory.

Hematologic improvement continued for 5 months after discontinuation of chemotherapy, with persistent normal white blood cell and platelet counts and normalization of hemoglobin. Fourteen months after the original presentation, the patient developed fever and thrombocytopenia. The white blood cell count was 8.4×10^3 per microliter, hemoglobin was 12.9 g per deciliter, and platelets were 78,000 per microliter. Bone marrow aspiration and biopsy revealed near total replacement by immature monocytoid cells and mature hemophagocytic histiocytes. Cytogenetics performed on the bone marrow showed 46, XY[9]/46, add/X(q22)Y, add(7)(p15), add (8)(p21), add (15)(q26)[cp11]. A blood specimen sent to the research laboratory at this time showed an MCSF level of 5.78 ng per ml, with control of 1.75 ng per ml. The specimen sent at the time of completion of chemotherapy 7 months earlier was also measured at this time and showed an MCSF level of 1.25 ng per ml, with the same control of 1.75 ng per ml.

The patient received broad spectrum antibiotics, including coverage for *Staphylococcus aureus*, which grew from an admission culture of an indwelling venous access device. The patient expired suddenly on the fourth hospital day, before receiving any antineoplastic therapy. Postmortem examination confirmed the bone marrow findings and showed extensive systemic involvement, with tumor in the lungs, liver, spleen, lymph nodes, kidneys, adrenals, and pituitary capsule. There was no evidence of myocardial infarction, pulmonary thromboembolism, or cerebrovascular accident.

MATERIALS AND METHODS

Human granulocytes and monocytes for cellular analyses were obtained from patient volunteers at University Hospital (State University of New York at Stony Brook) who had given their informed consent, as well as a single patient diagnosed as having histiocytic medullary reticulosis (HM). Blood was obtained via central venous access in that patient. Cells were separated by the standard Ficoll-Hypaque method as noted elsewhere in detail [9,10,14]; they were then washed three times in RPMI medium (RPMI, 25 m Hepes, Grand Island Biological Co., Grand Island, NY) and used for subsequent analysis.

Analysis of Cellular Activity

The analysis of chemotaxis was determined as noted extensively elsewhere [9,10,15]. Briefly, cells were placed at the extreme right of a petroleum jelly ring in saline in 100-μl volumes on a slide, and then covered with a glass cover slip. At the right edge of the petroleum jelly ring, an opening was made and a 100-μl (in physiological saline) volume of either DAMA or f Met-Leu-Phe (fMLP) was added. The solution moves under the slide by capillary action [15]. Experimental measurements were initiated at this time.

Chemotaxis was differentiated from chemokinesis by determining the axis of cell alignment and subsequent movement of the cell parallel to the chemical concentration gradient, through the use of cell analysis software (American Innovision, Inc., San Diego, CA). About 20 to 32 activated cells were observed for each 400-μm viewing diameter, and four additional viewing diameters were observed per slide. The entire process was repeated three more times and the resulting mean of these individual means (±SEM) was graphed. The variation for individual readings was between 5 and 9%.

The mixture of human cells (separately) with morphine, an agent known to inhibit cell activation, chemotaxis, chemokinesis, and spontaneous activation [9,10,12], was analyzed by phase-contrast microscopy, using a Zeiss (Mornwood, NY) Axiophot Microscope in conjunction with a JVC time-lapse video recording system, for conformational changes. Human granulocytes and monocytes each were analyzed at 30 min. Activation is defined as changes in cellular conformation ranging from inactive-rounded to active-amoeboid. This state was determined by measurements of cellular area and perimeter and were mathematically expressed by use of the shape-factor formula of the American Innovision Analysis System or the Image Analytics (Hauppauge, NY) system as previously described [9,15–18]. The proportion of activated cells was determined as noted elsewhere [9,10,15,18]. Activated cells change their conformation in response to an inhibitory pharmacological stimulus; they become immobile and rounded [9–11,15,19].

Plasma was obtained from patient volunteers at University Hospital who had given their informed consent, after the study was approved by the institutional review board. Plasma was also obtained from the patient diagnosed with HM. The plasma from these samples, as well as the HM sample, were immediately frozen to −70°C. In order to determine if plasma obtained from control and the HM patient contained signal molecules capable of influencing immunocyte behavior it was exposed to naive control cells obtained from the Long Island Blood Center (Melville, NY).

Opiate Binding Analysis

Human monocytes obtained from the Ficoll-Hypaque centrifugation and subsequent washing were homogenized −70°C in 50 volumes of 0.32 M sucrose, pH 7.4,

at 4°C, by the use of a Brinkmann (Westbury, NY) polytron (30 sec, setting no. 5) [10]. The crude homogenate was centrifuged at 900*g* for 10 min at 4°C, and the supernatant was reserved on ice. The whitish crude pellet was resuspended by homogenization (15 sec, setting no. 5) in 30 volumes of 0.32 M sucrose/Tris-HCl buffer, pH 7.4, and centrifuged at 900*g* for 10 min. The extraction procedure was repeated one more time, and the combined supernatants were centrifuged at 900*g* for 10 min. The resulting supernatants (S1′) were used immediately.

Immediately prior to the binding experiment, the S1′ supernatant was centrifuged at 30,000*g* for 15 min and the resulting pellet (P2) was washed once by centrifugation in 50 volumes of the sucrose/Tris-HCl. The P2 pellet was then resuspended with a Dounce hand-held homogenizer (10 strokes) in 100 volumes of buffer. Binding analysis was then performed on the cell membrane suspensions.

Cytokine determinations were made by a commercial company (Osaka, Bethesda, MD) from numerically coded blind samples.

Statistics

Analysis was performed by using the Student's *t*-test to compare controls with drug-exposed cells, as noted in the text. Controls were run for each treatment so as to avoid individual variations due to spontaneous activation of the cells.

RESULTS

Control spontaneously active granulocytes and monocytes exhibit a non-directed migration path whereas cells exposed to a concentration gradient of D-ala$_2$, met enkephalinamide (DAMA; 10^{-11} M) or FMLP (1.0 U/ml) [12], aligned their axis (i.e., elongated and became parallel) [9,10,19] with the concentration gradient and moved toward the higher concentration of the chemical stimulus [15]. Both monocytes and granulocytes obtained from the patient diagnosed with MH did not exhibit chemotaxis when exposed to either agent; instead, they continued to move in a chemokinetic manner. Additionally, over 90% of both cell types from the MH patient were spontaneously active compared to immunocytes obtained from controls (n = 42), which characteristically exhibit a spontaneous activation level of only 6–9% (Fig. 1; $P < 0.001$) [9,10,14,15,19].

Given this high level of spontaneously activated immunocytes, we attempted to determine if they could be inhibited by morphine, which has been previously shown to downregulate immunocyte activity [10]. Figure 1 demonstrates that the opiate alkaloid (10^{-6} M), regardless of the concentration, did not inhibit the HM-associated high level of immunocyte activation including chemokinesis. Interestingly, after 6 months of therapeutic treatment, a re-examination of these cells from an extremely limited blood supply also revealed a high level of spontaneous activation (Figs. 2,3; for both types of cells the form factor was below 0.50 in 46% of the cells); however, this level was not as high as when examined for the first time (Fig. 1). Furthermore, at this time, morphine (10^{-6} M) was able to inhibit their spontaneous level of activation (Figs. 2,3). However, the morphine-induced immunocyte inhibition with the MH cells upon opiate exposure only lasted for 20 min (Figs. 2,3). This time period is much shorter than that observed normally for morphine (1–2 h) upon immunocyte exposure [10], indicating that an immunocyte abnormality was still present.

At this point, it was of interest to determine whether the cells were spontaneously active due to an alteration of an intrinsic cellular mechanism(s) or due to the presence of a stimulating agent(s) in the plasma. Cell-free HM

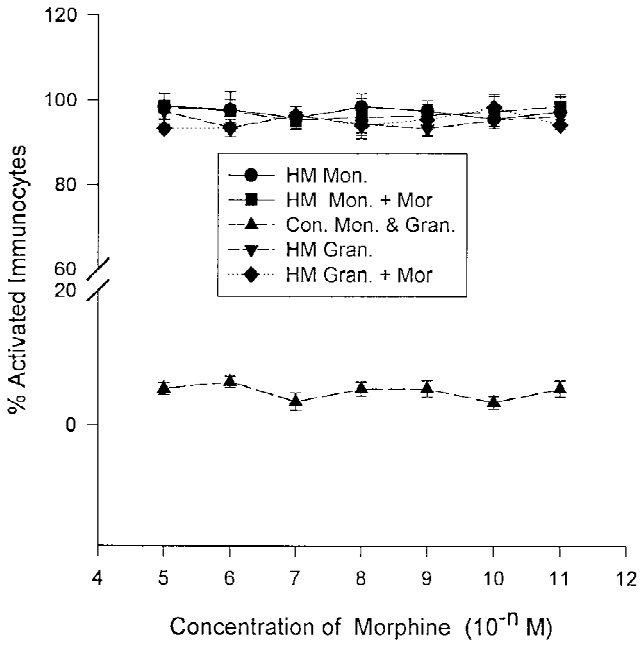

Fig. 1. HM monocyte and granulocyte activity and their responsiveness to morphine. HM monocytes and granulocytes were examined by image analysis for conformational change and mobility and compared to immunocytes from non-HM individuals. As indicated, the HM cells were significantly activated ($P < 0.001$). Furthermore, this high level of spontaneous activity could not be diminished by morphine (10^{-6} M), a known immunocyte downregulating signaling molecule. Each experiment involved measuring conformational change (cells exhibiting form factors below 0.5) and mobility of 32–44 cells in 4 fields of view (400-mm diameter), repeated 4–6 times and the mean value ± SEM graphed. Given the lack of difference between monocytes and granulocytes in measuring control and HM spontaneous activity, their values were combined for graphic representation. Statistical significance was obtained by comparison to non-HM-obtained cells by a one-tailed Student's *t*-test. Each test was run with its own separate control. Mon., monocytes; Mor., morphine; Gran., granulocytes.

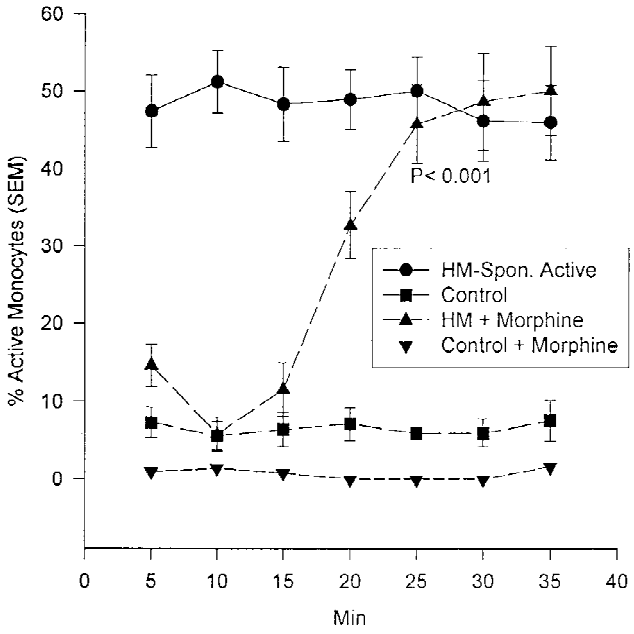

Fig. 2. HM monocytes' spontaneous activity and responsiveness to morphine were examined 6 months after the first evaluation. Details are as described in Figure 1. It can be observed that the spontaneous activation level is still high but not as elevated as observed in Figure 1. Additionally, morphine (10^{-6} M) appears to exert an immunocyte downregulating action that only lasts for 20 min compared to normal cells where it can last from 1–2 h. These experiments are limited in regard to replicates (3×) with approximately 35 cells per trial due to limited supply of HM samples given the patient's health status. $P < 0.01$ in the comparis on of the spontaneous level of activation noted here and that of non-HM controls and HM monocyte values found in Figure 1.

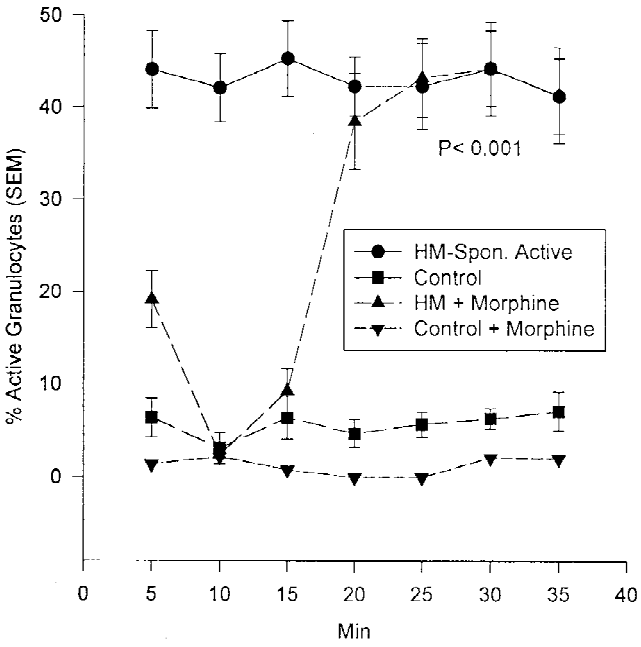

Fig. 3. HM granulocytes' spontaneous activity and responsiveness to morphine were examined 6 months after the first evaluation. Details are as described in Figure 1. It can be observed that the spontaneous activations level is still high but not as elevated as observed in Figure 1. Additionally, morphine (10^{-6} M) appears to exert an immunocyte downregulating action that only lasts for 20 min compared to normal cells where it can last from 1–2 h. These experiments are limited in regard to replicates (3×) with approximately 31 cells per trial due to limited supply of HM samples given the patient's health status. $P < 0.01$ in the comparis on of the spontaneous level of activation noted here and that of non-HM controls and HM monocyte values found in Figure 1.

plasma (100 μl) significantly stimulated monocytes (37% ± 5.7 [SEM]; n = 6 trials) obtained from non-HM individuals whereas control plasma obtained from normal non-stressed individuals did not induce this higher level of activity (11.4% + 2.6 [SEM]; n = 5 trials) during the observation period. Interestingly, not only were the monocytes significantly activated (form factor = 0.50) over controls ($P < 0.005$) but they also exhibited a higher percent of cells that were activated in comparison to granulocytes (22.1 + 3.6 [SEM]; $P < 0.05$). Additionally, the slow onset of MH-plasma activation (becoming mobile and ameboid) resembled kinetic curves obtained in our laboratory with many cytokines, which occurs over a 30-min period (data not shown) [15]. This data suggest that the disorder may involve production of immune signal molecules resulting in stimulation of cellular activation [12,13].

An examination of the plasma from the HM patient revealed that MCSF levels were elevated upon first admission to the hospital, and again just before the patient died (Table I; 6.4 and 5.78 compared to a control range of 1–1.75 ng/ml). Interestingly, at the time of ''remission'' during the stage where the cellular spontaneous activation level was lowered, the level of MCSF fell into the normal range (1.25 ng/ml). Granulocyte colony stimulating factor was also elevated in this patient whereas the levels of TNF, SCF, GMCSF, and IL-1β were not (Table I).

TABLE I. Cytokine Levels in a Control and HM Patient*

Cytokine	C	HM	2nd time	3rd time
MCSF (ng/ml)	1.0	6.4	1.25	5.78
GMCSF (pg/ml)	<8	<8		
GCSF (pg/ml)	53.5	350.3		
IL-1b (pg/ml)	<8	<8		
TNF (pg/ml)	<16	30.3		
SCF (ng/ml)	1.1	1.4		

*Levels were determined by ELISA by a commercial company as noted in the text. Given the paucity of the sample and the HM patients' condition, we could not continue to evaluate for all the cytokines listed.

In previous experiments, we determined that monocytes/macrophages contain the opiate selective and opi-

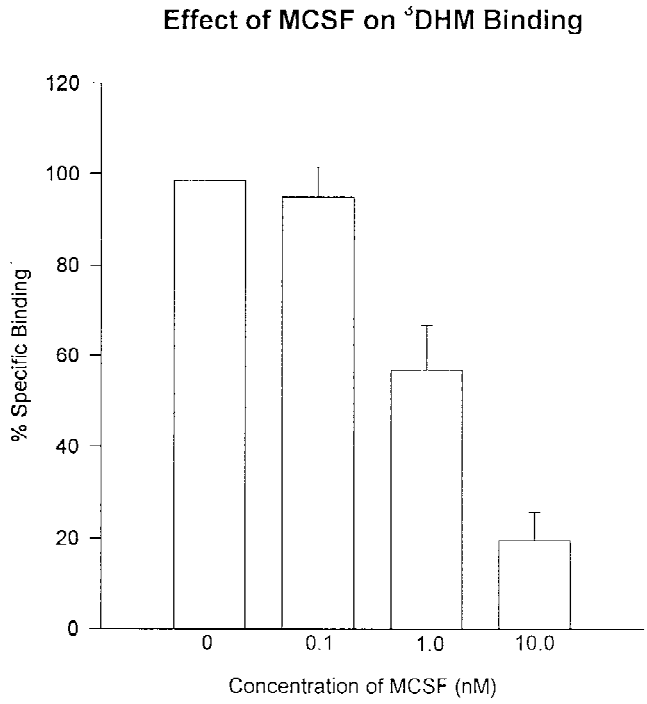

Fig. 4. Monocyte colony stimulating factor diminishes the density of the m_3 opiate receptor found on normal monocytes. One hundred percent binding is equal to 5 nM³ DHM bound to 149 fmol/mg protein. The binding experiment was performed only during the patient's first cellular analysis during this diagnosis period.

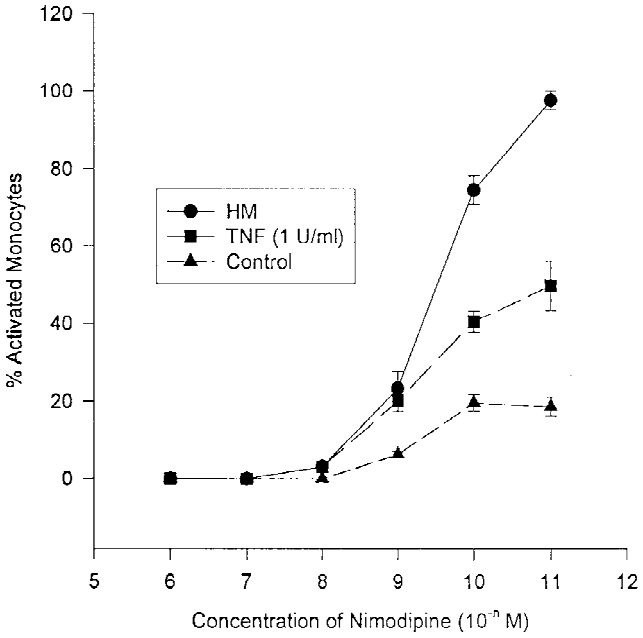

Fig. 5. Nimodipine diminishes the high level of spontaneous activation found in MH-obtained monocytes. This action of the calcium channel blocker is concentration dependent. The evaluation was performed only during the patient's first cellular analysis during this diagnosis period. Each experiment involved measuring conformational change (cells exhibiting form factors below 0.5) of 31–42 cells in 4 fields of view (400-mm diameter), repeated 4 times and the mean value ± SEM graphed. At 10^{-9} M nimodipine, $P < 0.005$ compared to the spontaneous level of activation noted in Figure 1 (97.3%).

oid peptide insensitive receptor, μ3 [10], which down-regulates the cells' activity and after exposure to activating cytokines. Given the initial lack and later a partial response to morphine's inhibitory action on the HM immunocytes, we examined monocyte membrane homogenates (limited supply for a one-point displacement analysis) for the presence of this receptor. Compared to control monocytes (5 nM ^{3}DHM bound to 149 fmol/mg protein), we could not detect the μ3 receptor on the HM monocytes, given the same level of protein per tube (0.12 mg). It should be noted that even though our supply of HM monocytes was quite small, in a comparable dilution of control cells we were able to detect opiate binding. Thus, we believe this finding is valid despite the small sample size due to the patient's status.

Simultaneously, and in a manner blinded to the above binding experiments, the HR plasma was evaluated as noted for the presence of varius signal molecules, i.e., cytokines. Based on the finding of elevated MCSF, we performed an in vitro incubation of MCSF with control monocytes (24 h, 37°C in RPMI at 5% CO_2) and subsequently the binding of ^{3}DHM to the membrane homogenates. We found that the μ3 receptor binding density was significantly reduced in a concentration-dependent manner (Fig. 4).

Given the general and yet specific role of intracellular calcium in the activation process of immunocytes including cytoskeletal mobilization [12,20], we examined the action of nimodipine a calcium channel blocker, for its ability to inhibit the HM-obtained monocytes (Fig. 5). Nimodipine, in a concentration-dependent manner, was able to inhibit chemokinesis and return the cells to a rounded-inactive conformation (Fig. 5). Cardiazem and verapamil also were effective in this regard (data not shown). In control cells, nimodipine also reduced the level of activation induced by tumor necrosis factor-α (Fig. 5). This result indicates that the HM immunocytes could obtain and use calcium from their external environment.

DISCUSSION

The present study demonstrates that: (1) HM monocytes and granulocytes exhibit a significantly high level of spontaneous activation and randomized movement; (2) these cells also fail to respond to potent immunocyte chemoattractant signal molecules under circumstances designed to induce chemotaxis; (3) they also fail to respond to signal molecules known to immobilize and in-

activate immunocytes; (4) the highly opiate selective and opioid peptide insensitive μ_3 receptor [10] could not be detected on monocytes obtained from the HM patient; (5) HM plasma contains a high level of MCSF; (6) MCSF exposed to control cells downregulates the μ_3 receptor density; (7) plasma obtained from the HM patient contains signal molecules capable of selectively activating control monocytes; and (8) the HM immunocytes can be inhibited or downregulated by calcium channel blockers, indicating the involvement and need for extracellular calcium in their hyper-activated state. Indeed, calcium channel blockers have been shown to have reversible inhibitory effects on the killing activity of both monocytes and granulocytes. This effect is thought to be mediated by a dampening of the respiratory burst process and not a reduction in phagocytosis [21]. Taken together, we conclude that monocytes and granulocytes obtained from this patient are hyperstimulated and do not respond to immunocyte downregulating signal molecules.

Monocyte colony stimulating factor, a hematopoietic growth factor, can be produced by monocytes, granulocytes, endothelial cells, and fibrocytes [22]. Its induction in these cells can also be achieved by a constellation of immune signal molecules. Interestingly, the role of the CSFs appears to be one of enhancing the activities of preexisting leukocytes as well as increasing the numbers of these cells [23]. In vivo ''priming'' of immunocytes with CSFs has been reported to increase their random migration [23], an observation with great significance in this study. In this regard, it has been surmised, as is the case with the integration of many chemical signal systems, that a balance exists between excitatory and inhibitory signals and that either a proliferative or a nonproliferative response will emerge based on this signal system homeostasis [24].

Recently, a greater understanding of monocyte activation and its regulation has emerged [25,26]. Briefly, signal molecules acting at surface receptors initiate second messengers. These second messengers presumably will then act through regulation of nuclear-binding proteins to stimulate or inhibit a particular pattern of gene expression. When these specific genes are expressed, their specific products will emerge in the cytosol and on the membrane and in the extracellular space. The result will be a monocyte state that is activated for a function; there is upregulation for that function and downregulation for others. In addition, activation of monocytes is regulated by the summation of stimulatory and inhibitory signals to which the monocyte is exposed. An on-signal usually has an opposing off-signal option thereby providing the necessary homeostatic balance that prevents host tissue damage. This observation is noted in the present study by the lack of a detectable opiate receptor, indicating a possible malfunction in the ''off'' mechanism.

Given the results of the present study, we can postulate that under normal circumstances the stimulatory signal provided by CSFs would be adequately counterbalanced by inhibitory receptor mechanisms. In the HM patient, we found elevated GCSF, which presumably was an unopposed stimulatory signal potentially responsible for granulocytic hyperactivity and lack of chemotaxis. We surmise these cells also exhibit undetectable levels of the $\mu 3$ receptor, which downregulates its activity via nitric oxide release [27], since they normally express it [19]. We also found elevated MCSF, which may have caused the same picture in the MH monocytes. We speculate, therefore, that only continuous immunocyte activation was possible, a result supported by the elevated serum level of MCSF. Furthermore, following a scan of cytokine levels in the serum, the elevated levels of MCSF support the present conclusion that it may be singularly important in this condition. Additional support for this hypotheses is found in the in vitro binding experiments, indicating the MCSF can decrease $\mu 3$ opiate receptor density on control monocytes that express this opiate receptor [10]. This may also serve to demonstrate that in in vivo conditions a balance exists between the stimulatory actions of MCSF and the immunoinhibitory actions of morphine, a potent and naturally occurring immunoactive substance [10,28].

Regardless of whether this is a case of elevated MCSF and G-CSF initially expressed from T-cell lymphoma cells, from malignant histiocytosis, or from biphenotypic cells, a number of conclusions regarding monocyte and granulocyte dysfunction in the malignant histiocytoses syndromes can be drawn from these results [12,29,30]. This patient may have had a malignant T-cell lymphoma, which produced the stimulatory cytokines (MCSF, for example) necessary to cause reactive hematophagocytosing histiocytosis and granulocytic hyperkinesis without chemotaxis; or he may have had malignant monocytoid histiocytosis, which produced cytokines (MCSF and G-CSF), resulting not only in the hemophagocytosing histiocytosis, but also granulocytic hyperkinesis without chemotaxis. He may also have had biphenotypic malignant immunocytes of macrophage and T-cell origin. The patient's history of Guillain-Barre syndrome may at first be taken as a clue to the primary cellular pathophysiology of his disease, especially since the Epstein-Barr virus (EBV), which is a causative agent in malignant histiocytosis, is also an etiologic virus in Guillain-Barre. As we have seen, however, the EBV genome may integrate into host T-cell lymphoma nuclei, as well as into histiocytic nuclei.

Part of the confusion stems from the similarities between malignant histiocytosis and the distinct benign reactive clinical syndrome Reiner and Spivak [31] have called hematophagic histiocytosis. Risdall and colleagues [32] were the first to actually describe a separate syndrome consisting of proliferating non-neoplastic histio-

cytes that were strongly hematophagocytic. Initially focused in response to viral infections, and thus termed by some VAHS, hematophagic histiocytosis has since been described as a reaction to almost any infectious pathogen, as well as to hematologic neoplasms and metastatic cancer in the absence of infection [31]. It can occur even in non-immunosuppressed hosts. Reiner and Spivak believe that malignant histiocytosis is over-represented in the literature, while hematophagic histiocytosis is actually more common than the literature would suggest. This may be understandable since the clinical and histological differences between malignant and hematophagic histiocytosis are not always clear-cut. Fever, lymphadenopathy, hepatosplenomegaly, and pancytopenia are clinical features of both syndromes. They share the same demographics (more common in middle age and in males) and while the prognosis in reactive hematophagic histiocytosis is more favorable, a rapid fatal outcome is still occasionally seen [33]. The presence of infection is not a reliable marker because of its high incidence in patients with hematopoietic malignancies. Malignant histiocytosis can complicate hematopoietic malignancies, but so can hematophagic histiocytosis.

Progress has been made in the last few years in using clonal markers for tumor cell lines as a way to distinguish between malignant and benign forms of histiocytosis. Unfortunately, immunohistochemical techniques have not helped characterize the origins of malignant histiocytosis. Three very recent papers underscore this point. Oka et al. [29] reported on three cases of malignant histiocytosis that were diagnosed on the basis of clinical symptomatology, a rapidly fatal course, and the morphology and growth pattern of both blastoid and hematophagocytic cells. The blastoid and hematophagic cells both expressed the phenotype Mac-387$^+$/KP1$^+$/lysozyme$^+$/polyclonal CD3$^+$, with the Mac-387, KP1, and lysozyme markers evidencing monocytoid cell origin and the CD3 marker suggesting T-lymphocyte origin. This result does not fully support the suggestion of Jaffe and colleagues that malignant histiocytosis may be accounted for by malignant lymphoma associated with lymphokine-stimulated non-neoplastic hematophagocytosing histiocytes [34]. It does suggest that, while many cases of so-called malignant histiocytosis have cells expressing only T-lymphocyte antigens, there are others that have a biphenotypic character stemming from macrophage and T-cell populations. Su et al. [35] recently presented evidence showing clonotypic proliferation of EBV genomes in the nuclei of large, atypical cells expressing T-cell markers in four patients with clinicopathologic features of malignant histiocytosis. They surmise that these are cases of T-cell lymphoma associated with cytokine-stimulated reactive histiocytosis. They also suggested that finding EBV genomes almost exclusively in the nuclei of atypical T-cells can distinguish malignant histiocytosis-like T-cell lymphoma from VAHS, in which EBV genomes are predominantly found in scattered B-cells, which then express the EBV antigens [36]. However, even here there is variation, for Ohshima and associates [37] recently reported a case of clinicopathologic malignant histiocytosis associated with chronic EBV infection. At necropsy, atypical hematophagocytosing histiocytes were noted to have extensively infiltrated bone marrow, liver, spleen, and lymph nodes. These cells were positive for monocyte markers such as a-1-antitrypsin and MA935, but negative for T-cell or B-cell markers. In addition, in situ hybridization studies revealed many histiocytic nuclei with EBV genomes. They suggest that the EBV genome may integrate into histiocytic host DNA, a situation akin to finding EBV genome in lymphoma nuclei, as noted above.

There is, therefore, evidence available for the malignant histiocytosis syndrome as secondary to (1) malignant T-cell lymphoma with reactive hematophagocytosing histiocytosis, (2) malignant monocytoid histiocytes, and (3) malignant biphenotypic immunocytes stemming from both the macrophage and the T-cell. Reactive hematophagocytic histiocytosis can be seen not only in response to T-cell lymphoma, but in reaction to other neoplasms, as well as to immunodeficiency states, autoimmune disorders, and to infectious diseases-as in VAHS [13]. It if of interest that the EBV has been associated with malignant histiocytosis of both the T-cell lymphoma subset, and true histiocytic varieties, as well as with reactive hematophagic histiocytosis.

In summary, MH may be regarded as a condition whereby the homeostatic balance controlling the activation state of immunocytes is diminished or not present. Supporting this conclusion is our finding that MH monocytes lacked the ability to bind opiate alkaloids. Thus, the high level of immunocyte activation noted in this condition could not be downregulated. In regard to the partial opiate alkaloid responsiveness of the monocyte during this patient's treatment period, we can conclude that the chemotherapy given did not alleviate the condition. However, it appeared to provide for a partial recovery, perhaps due to a reduction in MCSF stimulation and a consequent uncovering of μ3 opiate receptors. Furthermore, the in vitro demonstration of MCSF action in diminishing the opiate receptor levels points to that fact that in this condition the ability to regulate its synthesis may be damaged, leading to a lack of ''braking'' on immunocytes. Indeed, this condition may itself be related to the presence of the viral condition, possibly leading to disinhibiting MCSF expression. In retrospect, these findings also may provide a rationale for combining a calcium blocker with traditional chemotherapy in this generally fatal disease, given the in vitro inhibitory response of the MH immunocytes to calcium channel blockers.

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